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for "Research Reviews"
The Journal of the American Medical Association

Cancer and Viruses

When M. B. Shimkin[1], National Institutes of Health, Bethesda, Md., returned from a visit to Russia recently, he reported that at a meeting of the Academy of Medical Sciences in Moscow there was a lively discussion between the proponents and the opponents of the virus etiology of cancer. It is said that this is an important area of cancer research in the Soviet Union.

On this side of the Iron Curtain, the gap has narrowed between those who believe in the virus etiology of cancer and the advocates of the importance of "soil" susceptibility, chemical and other irritation, and genetically determined cell mutation. More important, there now appear also possibilities of applying laboratory observations to man.

"Soil" susceptibility is not only determined genetically. W. R. Bryan[2], National Cancer Institute, Bethesda, Md., has shown that in genetically similar individuals there may be a wide variation in susceptibility to one and the same tumor virus. There is also a wide variation in the susceptibility of different types of host to their respective specific tumor viruses as determined by the number of virus particles required to infect. The relation between tumor and virus is characterized by a diversity of interplay between virus quantity and level of host susceptibility in determining the nature and outcome of the host-virus interaction leading to malignancy. The same worker has suggested[3]

that serious consideration should be given to the metabolism of phospholipids in host-virus interaction. He believes that if oxidation products of phospholipids function in living cells as one of the defensive reactions against tumor viruses, chemical carcinogens, in solution in the lipid components of cells, might exert a sparing action on virus particles or might even favor their multiplication. This would form a common ground for the study of factors in carcinogenesis by chemical carcinogens and tumor-inducing viruses.

"Soil" susceptibility to viruses is influenced by age and by hormones.

Variations in susceptibility due to age have been reported for example by J. W. Beard[4], Duke University School of Medicine, Durham, N. C. Chicks are most susceptible to the virus of myeloblastosis at the age of three days and resistance increases rapidly with age, but they are far less susceptible to erythroblastosis at the three-day age than at 77 days. According to C. M. Southam, Memorial Center for Cancer and Allied Diseases, New York, chicks under one week of age - as judged by viremia - are highly susceptible to infection by West Nile, Japanese B, and related viruses; after three weeks of age, they are resistant to these viruses. Coxsackie virus is lethal to suckling mice, but causes no apparent ill effects in adult mice. The virus of lymphocytic choriomeningitis is more pathogenic in adult mice than in suckling mice. Thus the connection between age and virus susceptibility is not uniform. L. Gross[5], Veterans Administration Hospital, Bronx, N. Y., reported that

mice are only susceptible to the experimental inoculation of a filterable leukemia producing agent during the first few hours after birth and that they become resistant subsequently. S. O. Schwartz[6], Cook County Hospital, Chicago, believes that it is not necessary to use new-born animals for inoculation to produce leukemia. In Germany[7], it was found that adult mice were susceptible to cell-free tumor filtrates and developed myeloid leukemia.

Age is not only pertinent to "soil" susceptibility. In visceral lymphomatosis, a poultry disease wide-spread in the United States and thought to be caused by a virus[8], B. R. Burmester[2], U. S. Department of Agriculture, Agricultural Research Service, East Lansing, Mich., has shown that young hens shed significantly greater amounts of virus into their eggs than older hens.- F. Duran-Reynals[9], Yale University School of Medicine, New Haven, Conn., has pointed out since 1942, that not only the age of the recipient animal but also the age of the animal supplying the tumor is important in producing variation in tumor viruses; for example, the adaptation of a chicken tumor to the duck was accomplished only when, among other factors, the tumor-bearing chickens were of a precisely correct age: when they were too young or too old, the results were negative.

The same worker[4] has stated that quiescent viruses can cause cancer in experimental animals adequately treated by exposure to hormones and to chemical or physical carcinogens. He also reported that viruses not usually linked with tumor

growth may under given circumstances be implicated in the development of tumors: benign and malignant tumors have been produced at the site of vaccination with vaccinia virus in the skin of Swiss mice prepared by cortisone and methyl-cholanthrene, a chemical carcinogen. He believes that the dermal intercellular cement substance in the skin of Swiss mice forms a local barrier to viruses, bacteria, cancer cells and other agents. Hormones influence this protective action: estradiol benzoate promotes a rapid increase of dermal ground substance shown by thickening of the skin of the flanks, while cortisone has the opposite effect with thinning of the skin[10]. J. Meites and R. F. Langham[11], Michigan State University, East Lansing, have reported that feeding thyroxine, 2mg/kilo, significantly reduced the number and average size of benign papillomas and carcinomas developing in the skin of female albino mice after painting with 9, 10-dimethyl-1,2 dibenzanthracene and weekly applications of croton oil in liquid paraffin. Thiouracil, 2 gm/kilo, had the opposite effect.

The Mouse Mammary Tumor Agent (MTA) studied by J. J. Bittner[4], University of Minnesota Medical School, Minneapolis, only produces cancer readily in genetically susceptible strains after adequate hormonal stimulation. An interplay of Mammary Tumor Agent, genetic susceptibility and hormonal stimulation, therefore, decides whether tumors are produced; a smaller amount of one of these three factors may be compensated by an increase in one or both of the other two factors. Stimulation of the genetic factor can be obtained by selective inbreeding or proper hybridization,- hormonal stimulation by the use of natural or synthetic estrogens. It is assumed that the

presence of the Mouse Mammary Tumor Agent is essential, although it has been stated by investigators that in susceptible inbred strains of mice, breast tumors can be produced in the absence of demonstrable virus by intensive hormonal stimulation or by exposure to chemical carcinogens or X-rays.

V. Menkin[12], Temple University School of Medicine and Hospital, Philadelphia, has described an endogenous growth-promoting factor that is liberated from cells, mildly injured by such means as chronic inflammation, viral infection or hormone imbalance. He believes that in an appropriate genetic soil, this growth-promoting factor favors the development of neoplasia, but that in the absence of genetic susceptibility it does not induce neoplasia.

The introduction of the concept of "transduction" has done much to narrow the gap in our understanding of the relation between genetically determined cell mutation and viruses. As J. Lederberg[2], University of Wisconsin, Madison, put it: "The hallmark of a virus has been thought to be infectivity, or transmission from cell to cell through a medium. The discovery of transduction has largely obliterated this distinction." Transduction is the conferring of specific genetic traits upon the host cell by infecting phage[13]. Bacteriophages are viruses that reside in many bacteria. Some of them have remained unsuspected for a long time. The K-12 strain of Escherichia coli, for example, had been known for some twenty-five years before it was appreciated that it concealed a virus, now named lambda. In cultures grown from single mammalian cells, T. Puck, University

of Colorado Medical School, has recently been able to detect intracellular viruses by their characteristic effect on a sensitive "indicator" clone of cells when they were liberated by appropriate means. Thus a virus may be present in higher animals, as in bacteria, as a mutated version of a normal cell constituent. Mutagens convert such constituents into virulent viruses that destroy the cells containing them. A variety of environmental conditions are known to enhance such induced "mutation". It may be mentioned here that mutagens are known that are carcinogenic, and some carcinogens are known to be mutagenic.- In an ordinary culture of K-12 Escherichia coli, the free virus lambda is released by about one in every million bacterial cells; but, as Lwoff has shown, this release can be enormously accelerated by treating the bacteria with ultraviolet light[14]. There are a number of other means of releasing phages from their host bacteria experimentally. When phages released, for example, from lysed or ruptured bacteria of the genus Salmonella are allowed to infect other Salmonellae, they either destroy them,- like typical lethal parasites,- or they re-enter their "latent" form, conferring on their new host genetic traits of their previous bacterial host,- transduction.

Earlier studies of the mechanism involved in the "transformation" of "rough" avirulent pneumococci to "smooth" virulent ones had focussed attention on the role of deoxyribonucleic acid (DNA)[15]. On the basis of currently available experimental data, it is believed that DNA is the "transforming" substance. It has been shown that DNA can also transmit other

characteristics; for example, in the pneumococcus and in hemophilus influenzae resistance to streptomycin can be transferred from resistant donor bacteria to non-resistant ones by DNA. This DNA-transmitted resistance becomes a genetic characteristic of the new bacterial recipient. While in these cases DNA alone is the transmitting substance, the transducting bacteriophage in Salmonella is thought to consist of DNA surrounded by protein. When the virus has attached itself to the surface of the new bacterial host by means of its protein appendage, "the DNA nucleus of the phage .... enters the bacterium leaving the phage skin outside."[14]

Tobacco mosaic virus consists of ribonucleic acid (RNA) and protein. H. L. Fraenkel-Conrat[16], University of California, Berkeley, and G. Schramm[16], Max Planck Institute for Virus Research, Tübingen, Germany, have shown independently that its activity resides in its nucleic acid portion. Ribonucleic acid molecules on entering a cell may decisively influence intracellular events and the genetic apparatus[17]. An answer to the question how a great variety of biological information can be stored in nucleic acid molecules may lie in their chemical structure. The molecule of nucleic acid consists of nucleotides. With a molecular weight of around 300.000, a molecule of nucleic acid may contain about 1000 nucleotides. Each nucleotide consists of phosphoric acid, sugar (D-ribose in RNA, 2-deoxy-D-ribose in DNA) and four bases: adenine, guanine, cytosine, uracil in RNA; adenine, guanine, cytosine, thymine in DNA. A thousand unit nucleotide chain containing a coded repeat of these four

bases in the same ratio, for example, as they are present in tobacco mosaic virus nucleic acid, could form about 10^{590} different arrangements. A one hundred unit nucleotide chain of this composition could exist in about 10^{57} different arrangements. W. M. Stanley[18], University of California, Berkeley, has pointed out that this is a vastly larger number than the total of all living things. In his view, such a structure could therefore well carry the code for every bit of life on earth and in the sea.

When a normal cell becomes a cancer cell, it is thought that there are changes in the structure of nucleic acid in the cell. Compounds have, therefore, been studied that may act as antimetabolites for the four bases in nucleotides. One of them, 5-bromouracil, incorporated in a bacterial virus in place of thymine produced "the highest percentage of mutants ever recorded".[18] Nucleic acid metabolism is now being studied at many centers. It is coming into focus more and more in the study of the etiology and chemotherapy of cancer, in genetics and in virology.

Many hitherto unknown viruses have been found recently to be present in man[19]. Their clinical significance is unknown. Some may persist for years, perhaps for a life-time. Whether and how any of them are etiologically linked to cancer has not been established.- Advances in electron microscopy and modern methods of preparing ultra-thin sections have made it possible to study viruses in some detail. For the use of the electron microscope it is an important prerequisite to be able to cut consistently good thin sections.[20] It is now possible to cut sections about 0.02/µ thick. This means that a leukocyte, for

example, may be sliced into 800 sections [21]. At a recent meeting, P. O'B.Montgomery and his colleagues[12], University of Texas Southwestern Medical School, Dallas, reported on the use of the ultraviolet flying spot microscope for the study of living human cancer cells. With this microscope, developed by the group in Dallas in collaboration with the Philco Corporation of Philadelphia, the observer is able to study living cells for nine hours and longer, without damage to the cells and to take time-lapse motion pictures.

At another meeting[2], L. Dmochowski and C. D. Howe, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, exhibited photographs of virus-like particles in the cervical lymph nodes of a patient suffering from acute lymphatic leukemia. These particles appeared indistinguishable from the virus-like particles that produce experimental leukemia when injected into mice and chicken.

S. O. Schwartz and his colleagues[22], Cook County Hospital, Chicago, have produced leukemia in mice with filtrates obtained from the brain of patients who died of acute leukemia. The cell-free filtrate was injected into non-susceptible Swiss mice. At the end of 72 hours, they were killed, the brain was harvested and a new filtrate was prepared. This filtrate was inoculated into similar non-susceptible Swiss mice and the procedure was repeated. After five such passages, the final filtrate was injected into a different strain: AKR mice. Of 160 injected animals 115 developed leukemia. Heat inactivated filtrates and filtrates from non-leukemic brain similarly passed

were injected as controls. None of the 180 animals injected with these filtrates developed leukemia. Since activity of the filtrate was significant after serial passage through several animals of a heterologous strain of mice that would in theory dilute the original material to less than 10^{-13}, while no activity could be demonstrated when the original material was diluted to 10^{-5}, the assumption had to be made that only self-perpetuation could offer a satisfactory explanation.

All that can be said with certainty at present, is that some tumors in animals can be produced experimentally by virus-like agents under appropriate conditions. At this time, however, failure to demonstrate viruses on extraction from tumors, can not be taken as evidence that these tumors are of non-viral origin. In reviewing the diversified efforts that are made to establish evidence for a causal relation between viruses and cancer, one can not help being impressed by the complexity of the factors that may be involved. The connection between cancer and viruses is now attracting increased attention in many research centers. It is as well to await further developments with an open mind.

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